

POLLINATION EXPERIMENTS IN THE *MIMULUS CARDINALIS*–*M. LEWISII* COMPLEX

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ABSTRACT.—Experimental sets of *Mimulus cardinalis* and *M. lewisii* plants were (1) exposed to pollinators and (2) shielded from pollinators at study sites in Red Butte Canyon and Big Cottonwood Canyon, Wasatch Mountains, Utah. The exposed plants produced 1,535 seedlings and the shielded plants only 1. Clearly, seed production is dependent upon cross-pollination. A few syrphid flies were observed visiting the flowers but no hummingbirds or bumble bees, although the latter two have been reported as the main pollinators of *M. cardinalis* and *M. lewisii*, respectively. No interspecific hybrids were produced even though the species are fully interfertile, indicating that pollinators are faithful to their species or that different parts of their bodies pick up and carry pollen to the two different species.

Theoretically, changes in flower color or morphology may lead to a change in pollinators. How great must these changes be to affect reproductive isolation and launch the different populations on divergent evolutionary paths? Before exploring this question, it is necessary to establish whether or not reliance upon different pollinators is effective in reproductively isolating sympatric populations.

The *Mimulus cardinalis*–*M. lewisii* complex of interfertile species and varieties appears to be an excellent group to use in investigating this latter question (Vickery 1978). The species and their various populations differ greatly in the degree of interfertility (Vickery 1978, Vickery and Wullstein 1987); however, the two populations used in this study are fully interfertile and produce numerous F_1 and F_2 hybrids when artificially pollinated (unpublished data). The F_1 hybrids are pink flowered, and the F_2 hybrid populations segregate 3:1, various tints of pink to various shades of red (Vickery and Olson 1956, and unpublished data).

Mimulus cardinalis has flower color morphs of red, red-orange, and yellow. Its corolla lobes are sharply reflexed along the corolla tube, the corolla tube being 5 mm or less in diameter and 30–33 mm long. The bilabiate, sensitive stigma is exerted 16–20 mm. The two pairs of anthers, exerted 12–15 mm, are closely appressed to the style, one below the

other and immediately below the stigma. When a hummingbird probes the flower for nectar, its forehead brushes the stigma and anthers, picking up pollen grains that may be deposited on the stigma of the next flower. *Mimulus cardinalis* is such a typical hummingbird flower that it was used as the cover illustration of Grant and Grant's (1968) book, *Hummingbirds and Their Flowers*.

Mimulus lewisii has flower color morphs of light lavender and deep magenta. Its corolla lobes are thrust forward in the light lavender-flowered race and are gently recurved in the deep magenta-flowered race. The corolla throat is open and approximately 10 mm wide by 7 mm high in the lavender-flowered race of the Sierra Nevada and approximately 12–15 mm wide and high in the magenta-flowered race of the Rocky Mountains. The corolla tubes are approximately 25 mm deep in both races. The sensitive, bilabiate stigma is included and is about 2 mm below the corolla orifice in the Sierran race. In the Rocky Mountain race the stigma is included but on a level with the orifice. The anthers occur in pairs, one below the other and 1–2 mm below the stigma in both races. *Mimulus lewisii* flowers are well adapted for bees landing on the labellum petal of the corolla and climbing into the flower for nectar and/or pollen. Their bodies brush the stigma and anthers and pick up pollen which they then may deposit on the

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stigma of the next flower. *Minulus lewisii* has textbook-typical bee flowers (Faegri and van der Pijl 1979).

Minulus cardinalis ranges from southern Oregon south to central Baja California, and from the California Coast Range inland to mid-elevations in the Sierra Nevada (Vickery and Wullstein 1987). The lavender-flowered race of *M. lewisii* occurs at elevations higher than *M. cardinalis* in the Sierra Nevada. The magenta-flowered race ranges from the northern Sierra Nevada north to Alaska and east to the Rocky Mountains (Vickery and Wullstein 1987). The two species rarely overlap and then only when seeds of *M. lewisii* wash down into the range of *M. cardinalis* and become established as ephemeral populations on streamsides, principally in the central Sierra Nevada (Hiesey et al. 1971). The sympatric populations flower at the same time, which heightens the importance of their reproductive isolation by different pollinators.

Both species produce nectar throughout the day, although the nectar production of *M. cardinalis* is far more copious than that of *M. lewisii*.

Before the main, long-range question of the effect of differences in flower color and/or shape on the pollinators can be investigated, it is necessary to establish some basic facts. First, do *M. cardinalis* and *M. lewisii* require the service of pollinators? Or, do they self-pollinate, at least to some extent? Second, if pollinators are required, which ones normally visit the flowers of the two species? Once the norms are ascertained, then the effect of different colors and/or shapes can be determined. Third, are the pollinators faithful to their species? Or, does cross-pollination occur between the two species? That is, would a difference in pollinators isolate the two species reproductively? Or, only partially? Or, would the differences between the species tend to swamp out? The purpose of this study is to answer these intrinsically interesting basic questions and, in addition, to provide the necessary foundation data for the long-range study.

MATERIALS AND METHODS

Plants of typical red-flowered *M. cardinalis* Douglas (culture I3313 from Cedros Island, Baja California) were grown from seed in the

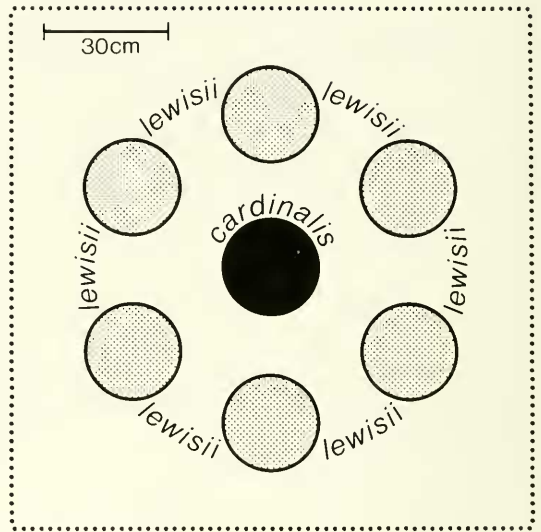


Fig. 1. Arrangement of potted plants in the experimental sets. The reciprocal arrangement was of *M. lewisii* in the center surrounded by six *M. cardinalis* plants. The dotted line indicates the location of the screen cage in the pollinator exclusion trials.

University of Utah greenhouse, as were plants of magenta-flowered *M. lewisii* Pursh (culture 5875 from Alta, Utah), typical of the Rocky Mountain race. The seedlings were transplanted first into 4" pots and then, when large enough, into deep 8" pots. The bigger pots allowed the plants to grow larger (20–60 cm high) and produce many flowers for the field studies.

The field tests were carried out at two sites in the Wasatch Mountains of Utah. The first location was in the Red Butte Canyon Natural Area, Salt Lake County, and the second, at Silver Fork in Big Cottonwood Canyon, also Salt Lake County. In Red Butte Canyon the pots of plants were placed on the wet delta at the head of the reservoir, elevation 5,360 feet, so they could be watered naturally. At Silver Fork the pots of plants were placed in the meadow, elevation 7,800 feet, below Silver Fork Lodge, and were watered daily by Luther Light.

The plants were arranged in experimental sets of seven plants. In each set the center pot contained a plant of one species, e.g., *M. cardinalis*; and a whorl of six pots surrounding it each contained one plant of the other species, e.g., *M. lewisii* (Fig. 1). This arrange-

ment was designed to facilitate cross-pollination, should it occur.

At the Red Butte Canyon site, four experimental sets were exposed to the pollinators. Two sets had *M. cardinalis* as the center plant surrounded by *M. lewisii* plants, and two sets had *M. lewisii* in the center surrounded by *M. cardinalis*. In addition, four corresponding sets were placed in 1 × 1 × 1-m screen cages (plastic mesh, 20 threads per inch, pore size 1 × 1 mm) designed to exclude pollinators. The same experimental design was repeated at the Big Cottonwood Canyon study site. The first study site was in a streamside, partially shaded, maple-box elder forest; the second was in an open meadow in the aspen-spruce forest. Two contrasting sites were employed as controls in case different pollinators occurred in different habitats and at different elevations in the canyons.

At the beginning of the experiments all capsules and flowers were removed. New flowers began opening the next day. The plants were observed to note pollinator visits for a total of 20 hours for each experimental set. The observations were one-hour periods scattered from dawn to dusk on different days. Experiments were run for one month, by which time new flowers had opened on most plants; they had been exposed to pollinators (that is, the uncaged sets); and capsules had formed and were starting to ripen. Plants were then returned to the greenhouse, and capsules on plants of both exposed and shielded sets were harvested as they ripened. Seeds set were not counted inasmuch as the number of seedlings produced seemed a more meaningful measure of pollinator success or selfing rate.

In the summer of 1984 all seeds produced by the peripheral whorl of plants in each experimental set were sown together in one pot, and seeds produced by the plant in the central pot were sown in another. Resulting seedlings were scored as to whether they were of parental type, indicative of pollinator faithfulness, or hybrids, indicative of pollinator promiscuousness, that is, pollinators visiting both species. The F₁ hybrids, which have leaves intermediate in width between the broad leaves of *M. cardinalis* (13013) and the narrow leaves of *M. lewisii* (5875), can be distinguished at an early stage. Nevertheless, the seedlings were grown until they flowered and exhibited either the unambiguous F₁ pink

color or the parental red (*M. cardinalis*) or magenta (*M. lewisii*).

RESULTS AND DISCUSSION

Are pollinators necessary? Results of this research indicate a resounding yes! All plants in cages set a total of only one seed that germinated and grew into a seedling (Table 1). It was a vigorous *M. cardinalis* plant from the central plant in one of the Red Butte Canyon sets. In contrast, plants in the sets exposed to pollinators produced a total of 1,535 seeds that germinated and grew into seedlings. Of these, 1,047 were *M. cardinalis* and 488 were *M. lewisii*. While there were equal numbers of plants, there were more *M. cardinalis* flowers. Hybridizations were possible in three of the eight experimental sets. The results are very clear despite the heavy depredations by deer and the lack of flowering in the other sets (Table 1).

Pollinator observations revealed the presence of Broad-tailed Hummingbirds and bumble bees at both sites and syrphid flies at the Red Butte Canyon site. Hummingbirds and bumble bees flew near the *Mimulus* plants at both sites but, surprisingly, were not observed visiting the flowers. However, in the Red Butte Canyon experiments, small syrphid flies visited both species occasionally, but not on the same foraging bout (1–5 minutes, 1–3 flowers) nor often enough to account for the observed seed sets. There were only five total visits (at scattered times), and the only pattern revealed was that syrphids visited the lower-elevation experiments of Red Butte Canyon but not the higher-elevation experiments of Big Cottonwood Canyon. The flies appeared to be foraging for pollen inasmuch as they walked all over the flowers, including the anthers and pistils.

Of the 1,535 seedlings produced, not one was a hybrid. This was true also in the progeny grown from plants of a natural, sympatric population of both species in the Yosemite Valley by Hiesey et al. (1971). Apparently the pollinators are effectively faithful to each species both in the Wasatch Mountains and the Sierra Nevada.

The study raises some intriguing questions. Why were hummingbirds and bees not observed pollinating the flowers when the Carnegie study (Hiesey et al. 1971) showed

TABLE 1. Seedlings produced from the seeds set by *M. cardinalis* and *M. lewisii* plants in Red Butte Canyon and Big Cottonwood Canyon (1) when exposed to pollinators and (2) when shielded from pollinators by cages. Plants were arranged in sets consisting of a center plant of one species surrounded by a whorl of six plants of the other species (see Fig. 1).

Set number	Composition of set	Number of seedlings resulting	
		Exposure to pollinators	Shielded from pollinators
Red Butte Canyon experiments			
#1	1 central <i>cardinalis</i> 6 peripheral <i>lewisii</i>	0* 0*	1 <i>cardinalis</i> 0
#2	1 central <i>cardinalis</i> 6 peripheral <i>lewisii</i>	0* 71 <i>lewisii</i>	0 0
#3	1 central <i>lewisii</i> 6 peripheral <i>cardinalis</i>	190 <i>lewisii</i> 350 <i>cardinalis</i>	0** 0**
#4	1 central <i>lewisii</i> 6 peripheral <i>cardinalis</i>	0** 420 <i>cardinalis</i>	0 0
	Total <i>cardinalis</i> seedlings	770	1
	Total <i>lewisii</i> seedlings	261	0
	Total F ₁ hybrid seedlings	0	0
Big Cottonwood Canyon experiments			
#5	1 central <i>cardinalis</i> 6 peripheral <i>lewisii</i>	184 <i>cardinalis</i> 137 <i>lewisii</i>	0 0**
#6	1 central <i>cardinalis</i> 6 peripheral <i>lewisii</i>	93 <i>cardinalis</i> 90 <i>lewisii</i>	0 0**
#7	1 central <i>lewisii</i> 6 peripheral <i>cardinalis</i>	0* 0*	0 0
#8	1 central <i>lewisii</i> 6 peripheral <i>cardinalis</i>	0* 0*	0 0
	Total <i>cardinalis</i> seedlings	277	0
	Total <i>lewisii</i> seedlings	227	0
	Total F ₁ hybrid seedlings	0	0
	Grand total <i>cardinalis</i> seedlings	1,047	1
	Grand total <i>lewisii</i> seedlings	488	0
	Grand total F ₁ hybrid seedlings	0	0

*Capsules on experimental plants eaten by deer.

**Failed to flower during experiment

them to be the main pollinators of *M. cardinalis* and *M. lewisii*? What would their visits show about temporal partitioning? Or, perhaps, morphological partitioning for pollen transfer on different parts of the pollinator's body? Are there significant differences in quantity and sugar content of the nectar produced by the flowers that might affect pollinator preferences and visits?

In conclusion, despite the questions raised for future studies, these experiments demonstrated that neither *M. cardinalis* nor *M. lewisii* self-pollinates under natural conditions; at least, the rate is less than .1%. Clearly, pollinators are required for seed set. Only syrphid flies were observed actually pollinating the flowers, although hummingbirds and bumble bees are probable pollinators also (Hiesey et al. 1971). The experiments showed

that the pollinators (seen and unseen) are effectively faithful to their own *Minulus* species. So, (1) pollinators are required, (2) the only observed pollinators are the small syrphid flies, and (3) the pollinators are effectively faithful to their species, either on each foraging bout or by using species-specific parts of their bodies for pollen transfer.

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